DARK ADAPTATION AND REVERSAL OF PHOTOTROPIC SIGN IN DINEUTES

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Introduction

Animals which exhibit orientation and directed movement with respect to a light source are said to be positively or negatively phototropic, according as they move towards or away from the source. Under certain conditions, a change of phototropic sign may occur, e.g. an animal which normally moves towards the source will move from it. Among the more important factors which have been shown to produce such a reversal of phototropic sign are: difference in absolute light intensity, change of light intensity, temperature, pH, and some chemical substances. The possible effect on phototropic sign of the degree of dark adaptation, however, has been largely neglected. Crozier and Wolf (1928) demonstrated the effect of dark adaptation on the strength of phototropism in *Agriolimax* and more recently Wolter (1936) has reported that some specimens of *Carcinus maenas* show a change of phototropic sign with dark adaptation. Since Clark (1931, 1933) demonstrated the process of dark adaptation by means of phototropic reactions for the "whirligig beetle" (*Dineutes assimilis*), it was decided to use this beetle in testing for the possible effect of dark adaptation on the sign of phototropism.

Material and Methods

The animals used in these experiments were of the species *Dineutes horni*,[1] and were collected from Fresh Pond, Cambridge, Massachusetts. They were kept in an aquarium in a lighted room, and were fed on pieces of fresh meat and fish, floated on wood.

The eye in *Dineutes* is divided into dorsal and ventral parts on each side, and the phototropic responses were compared when the whole, and when only part of each eye was functional. Blinding was effected by blackening the surface of part of the eye with asphaltum varnish.

In each experiment the beetle was first light-adapted and was then left for the desired period in a covered vessel of water. Following this

[1] I am indebted to Mr. C. Parsons of Harvard University for the identification of this species.

period in darkness the phototropic reaction was observed by allowing the animal to crawl on a dry, horizontal test-plate of ground glass. This was illuminated from one side by a diverging beam of light emanating from a slit ($1\frac{1}{2}'' \times \frac{3}{4}''$) covered by a ground glass plate, behind which a shielded 100-watt lamp was set. The test plate was level with the bottom of the slit and rested on a dull black surface. A little light was diffusely reflected up from the surface of the test plate but the greater part of the light reached the insect directly from the horizontal beam. The light intensities at the two ends of the test plate were 1.8 and 0.6 f.c. respectively.[2] The path taken by the insect was followed with a pencil, and marked directly on the plate. This did not appear to disturb the beetle.

Light adaptation was effected by placing the beetle, in water in a glass cell ($2\frac{3}{4}'' \times \frac{3}{4}''$) for 20 minutes, in the path of a beam of light from a 500-watt projection lamp. A strongly-reflecting surface at the " darker end " of the cell ensured fairly uniform lighting. Intensity of adapting light was 4,800 f.c.[2] Heating was avoided during the light adaptation period by interposing heat-absorbing filters.

At the beginning of each experiment, the beetle was placed by means of a piece of stiff paper at approximately the centre of the test plate. It was not headed precisely in any special direction, as earlier experiments had shown that the animal oriented immediately, irrespective of the direction in which it was placed.

Observations

With all eyes functional, a strong, positively phototropic response was invariably obtained, whether the animal was light- or dark-adapted.

When any two, or when only one of the four eyes[3] was functional, a negative response was obtained if the beetle were dark-adapted. If only one eye was blackened the animal was photopositive. In the great majority of experiments, either the two upper, or the two lower eyes were covered. This avoided any complications, such as possible circus movements (cf. Clark).

It was often impossible to carry out all the experiments, with the various combinations of eyes, on the same individual, but the behavior was sufficiently constant to combine the results obtained from different animals. (A very few individuals were found in which the phototropic reactions were irregular.)

[2] Light intensities were measured with a Macbeth Illuminometer, using dense filters for the highest intensities.

[3] To obviate needless repetition, the upper and lower halves of the eyes are simply referred to in the results as upper and lower eyes.

The results of experiments with (*a*) two upper eyes only functional and (*b*) two lower eyes only functional were similar. The beetles were always positively phototropic when they were light-adapted. They were usually still positively phototropic after dark adaptation of less than two hours duration, but became negatively phototropic after dark adap-

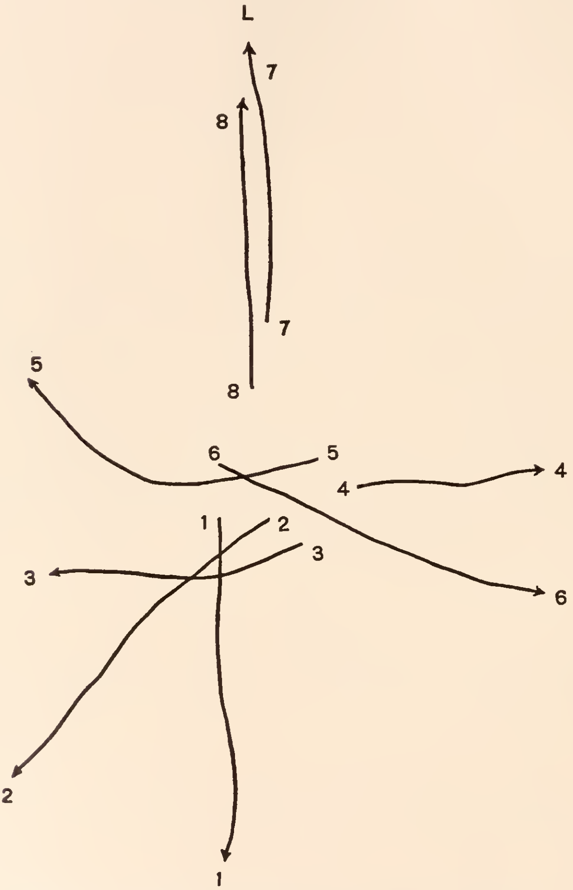

FIG. 1. Tracings of paths followed by *Dineutes* (Individual *X*) May 19, 1938, in 8 successive trials after dark adaptation for seven hours. The upper eyes were functional. The numbers refer to the order of trials. *L.* indicates light source.

tation of more than two hours duration. However, the actual period of dark adaptation necessary to bring about the change in phototropic sign showed considerable individual variation; in a very few cases, even one hour was sufficient.

A beetle which had been dark-adapted for a period sufficient to become negatively phototropic would show a reversal to the original positive condition as it became partially light-adapted. This was shown as follows. A beetle, after dark adaptation for many hours, was tested repeatedly in the beam. The responses exhibited at first were all photonegative, but the sign of phototropism became reversed after a certain

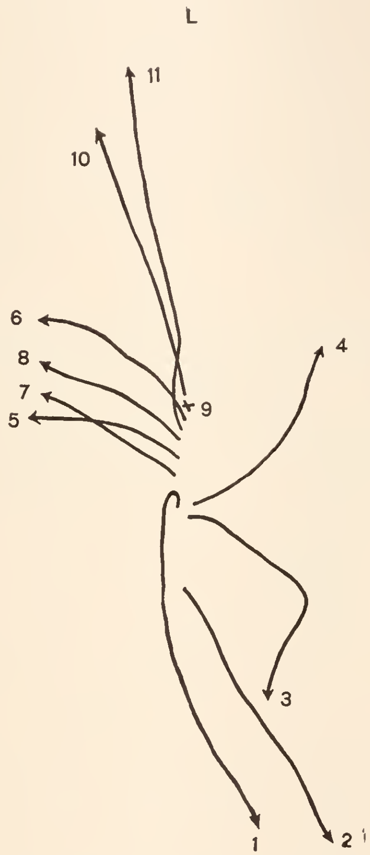

FIG. 2. Tracings of paths followed by *Dineutes* (Individual *B*) December 15, 1937, in 11 successive trials after dark adaptation for twelve hours. The upper eyes were functional. No " run " was obtained in Trial 9.

amount of light adaptation had been brought about by the light from the test beam itself. Subsequently, all further responses were consistently photopositive.

This reversal of phototropic sign with light adaptation was also observed, while avoiding the repetition of trials. A fully dark-adapted beetle was tested once to demonstrate the negatively phototropic reaction.

It was then left in the test beam, surrounded by a small glass cell, until the light effected a sufficient degree of light adaptation. A single new trial then showed the beetle to be positively phototropic.

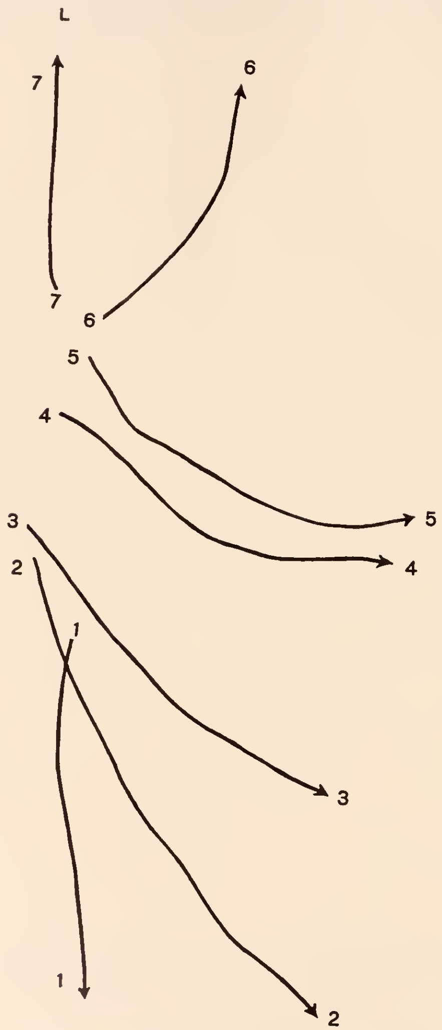

FIG. 3. Tracings of paths followed by *Dineutes* (Individual *V*) May 20, 1938, in 7 successive trials after dark adaptation for seven hours. The lower eyes were functional.

It was obvious that a considerable period of exposure to darkness was necessary to elicit the negatively phototropic reaction. Experiments were next carried out to test the possibility that with all eyes functional, a very prolonged period in darkness might cause a reversal of phototropic sign. The results of these experiments, with seven individuals, showed that reversal cannot be brought about by 24–65 hours of dark adaptation, if all eyes are functional.

If the two upper or the two lower eyes were blackened but a very few ommatidia of one of these eyes were left exposed, the beetle remained photopositive when fully dark-adapted. On completely covering the eye, the beetle, when dark-adapted, became negatively phototropic.

A few experiments were carried out with any three eyes blackened, and other experiments in which one upper eye and one lower eye of the opposite side were blackened. Such experiments, with any two or any three eyes covered, always showed that the dark-adapted beetles were negatively phototropic, and that light adaptation caused a reversal to positive phototropism.

TABLE I

Change in the direction of phototropic path followed by Dineutes (Individual V), in 7 successive trials, consequent upon light adaptation. The animal was first dark-adapted for 7 hours. The lower eyes were functional.

Trial Number	Angle of Deviation from Normal Positive Path
	degrees
1	180
2	150
3	135
4	110
5	110
6	50
7	0

Exact time of each trial, since beginning of exposure to test light was not recorded.

On the orthodox Loebian view, it would be expected that on repeatedly testing an originally fully dark-adapted beetle, a number of photonegative trials would be first obtained, and then, if a reversal of sign occurs with light adaptation, a sudden and complete change to positive phototropism would be observable. It was actually found, however, that an incompletely light-adapted beetle pursued a path *at an angle* to the light beam. If the direct negative response may be regarded as a deviation of 180° from the positive path, then with exposure to light, the angle of deviation gradually diminished until it approached 0° (i.e. the beetle was again positively phototropic).

It was possible to obtain such records from several individuals, and to repeat the observations on the same beetle. Although the actual paths (Figs. 1–3) were not straight, and in spite of some irregularities

Table II

Change in the direction of phototropic path followed by Dineutes (Individual B), in 6 successive trials, consequent upon light adaptation. The animal was first dark-adapted for 12 hours. The upper eyes were functional.

Trial Number	Total Time of Exposure to Test Beam	Angle of Deviation from Normal Positive Path
	minutes	*degrees*
1	0	180
2	1	120
3	3	75
4	8	60
5	18	(no "run" obtained)
6	38	20

in respect of angle, in general the results showed a surprisingly consistent trend.

The time required for complete reversal of phototropic sign varied from < 10 to > 30 minutes. It is believed that a rough estimate of

Table III

The effect of dark adaptation on the direction of phototropic path in Dineutes (Individual V). The lower eyes were functional.

Time in Dark Following Standard Light Adaptation	Result of Single Trial in Test Beam: Angle of Deviation from Normal Positive Path
minutes	*degrees*
5	5
15	35
24	90
30	(140)
45	120
90	160
180	180

the course of light adaptation can be obtained by measuring the successive deviation angles (Table I).

Table II shows an experiment conducted on a beetle which was dark-adapted for a longer period. The total duration of exposure to light from the test beam since the beginning of the experiment was also recorded for each trial.

If these results do really indicate the course of light adaptation, it should be possible, using similar methods, to follow the course of dark adaptation. To test this possibility, a number of experiments were carried out using different periods of dark adaptation. In each experiment the animal was first light-adapted by means of the usual adapting light for 20 minutes. It was then dark-adapted for a given period, and a single trial made in the test beam. By using various periods of dark adaptation a number of trials were obtained, and the results (e.g. Table III) showed that with progressively longer periods of dark adaptation, the paths pursued showed an increasingly greater deviation from the positive path. An approximate dark-adaptation curve has been constructed from these data (Fig. 4) for one individual.

The possibility that the change in the paths pursued might be caused by the repeated disturbance of the beetle when replacing it at the centre of the test plate was investigated. The test plate was constantly moved in such a way that a beetle which had been originally fully dark-adapted was kept in the beam without being replaced. The animal at first

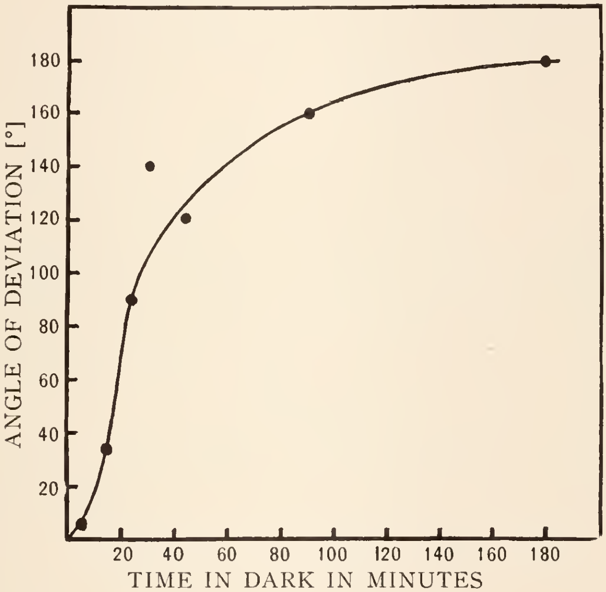

Fig. 4. Progressive deviation from the normal positively phototropic path during reversal of phototropic sign with dark adaptation, in *Dineutes horni* (Individual *V*). (The lower eyes were functional.)

moved from the light source, but with continued exposure it turned gradually towards the source until it reached the edge of the test plate. The changes in path are therefore not the result of disturbance, but do depend on the degree of adaptation.

Discussion

McIndoo (1929) states that Schmitt-Auracher believed there was a relationship between the state of adaptation and pigment deposition in insect ocelli and the sign of phototaxis. In the present observations on *Dineutes,* a reversal of phototropic sign could never be obtained when all four eyes were functional, but if *any* two, or *any* three, eyes were blackened, the fully dark-adapted beetles were always negatively phototropic. The difference in behavior may depend upon the area of photosensitive surface stimulated. Although one cannot compare human, subjective sensations with animal tropisms, it is interesting that experiments on the intensity discrimination of the human eye have shown that the use of a small test field of high intensity may cause uncomfortable glare and even pain, while with a larger test field of the same average intensity, vision is normal.

When only partially light- (or dark-) adapted, *Dineutes,* with only two eyes functional, moves at an angle to the light beam. Rádl, Carpenter, and especially Dolley (1916) and Clark (1931 and 1933) have discussed movement of phototropic insects at an angle to a beam of light, but in all such cases only *one* of a pair of symmetrical eyes was functional, and deviation might be then expected. Clark supposed that light from the direct beam, and light from the background acted on the functional eye. But in the experiments described, with both upper, or both lower eyes functional, light from both background and beam should act equally on the two sides, and therefore, according to Loeb, the insect should move *directly* to or from the source. Indeed, Clark states that if in *Dineutes* both upper or both lower eyes are blackened, the beetle moves straight towards the light. But in Clark's experiments the beetles were consistently photopositive, and provided *D. horni* is strongly positively phototropic, it moves straight to the source also.

Mast (1938) has shown that the phototropic reflexes in insects vary according to the region of the eye stimulated. In *Dineutes,* according to Clark, the posterior ommatidia are much more sensitive than the anterior ommatidia. Possibly then, during reversal of phototropic sign with light adaptation in *D. horni,* some of the ommatidia become light-adapted more rapidly and give rise to reflexes which are opposed by the less sensitive ommatidia. Morphologically symmetrical ommatidia also may not adapt at exactly similar rates, and therefore photochemical reactions will proceed at different rates on the two sides during partial adaptation, and a deviation would result. When *all* ommatidia are fully adapted, the beetle will move straight to the source.[4]

Although an exact explanation must therefore await further work, it is obvious that the simple Loebian theory will not account for the facts here presented. Light must act in a more complex manner, and among

[4] Some recent experiments on the related genus, *Gyrinus,* have shown that even with all eyes functional, a positively phototropic beetle, when it is fully dark-adapted, may pursue a path deviating widely from the normal straight photopositive path.

other factors, the phototropic responses must depend to a considerable extent upon the region of eye stimulated, as Mast has repeatedly emphasized.

Summary

1. When all eyes are functional, *Dineutes horni* is positively phototropic after dark or light adaptation.

2. With one or with two eyes functional, *Dineutes* is positively phototropic when light-adapted, but is negatively phototropic when fully dark-adapted.

3. At intermediate stages of dark and light adaptation, the beetle moves at an angle to the light rays. The courses of dark and of light adaptation were followed by a study of these "angles of deviation" from the incident rays.

4. Possible theories are discussed to account for these results.

ACKNOWLEDGMENTS

This work was carried out at Harvard University during the tenure of a Henry Fund Fellowship. The author wishes to thank Dr. G. L. Clarke, of Harvard University, for his constant advice and criticism throughout the investigation, and Mr. J. Armstrong, of Harvard, for his contribution to the discussion.

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